US006132979A

United States Patent [19]

Murakami

[11] Patent Number: 6,132,979

[45] Date of Patent: *Oct. 17, 2000

[54] CYTOTOXICITY TESTING METHOD

[75] Inventor: Toru Murakami, Tokyo, Japan

[73] Assignee: NEC Corporation, Tokyo, Japan

[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

[21] Appl. No.: 09/085,647

[22] Filed: May 27, 1998

[30] Foreign Application Priority Data

May 27, 1997 [JP] Japan 9-136886

[51] Int. Cl.[7] G01N 33/567

[52] U.S. Cl. 435/7.21

[58] Field of Search 435/240.243, 174, 435/176, 177, 178, 179, 180, 181, 182, 240.22, 240.23, 240.1, 226, 29, 721, 395, 305.1, 32, 11, 25; 424/574; 436/34

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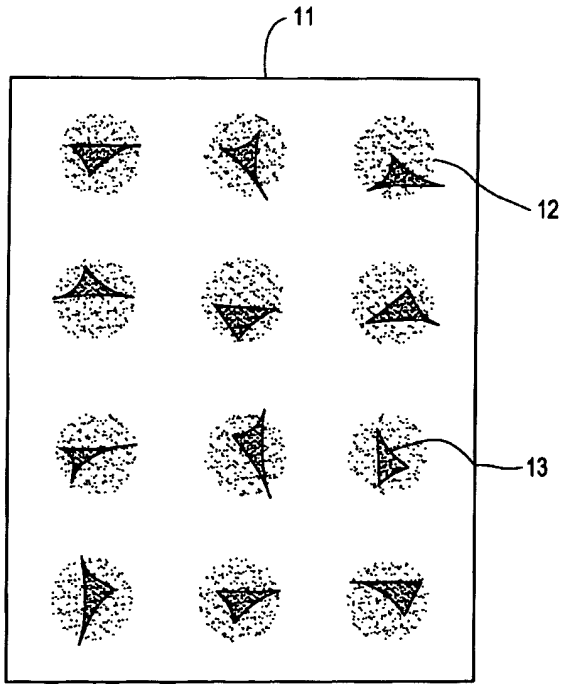
[57] ABSTRACT

A cytotoxicity testing method of the present invention allows live cells to be directly and accurately counted one by one in order to determine the survival rate of cells. This allows the toxicity of a chemical substance to be quantized with high accuracy.

8 Claims, 2 Drawing Sheets



DOCUMENT-IDENTIFIER: US 6132979 A
TITLE: Cytotoxicity testing method

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BSPR:
A tenth cytotoxicity testing method determines whether cultured cells are alive
or dead by flow cytometry. When a Trypan Blue solution is added to a cell
solution, only deal cells take it thereinto. Trypan Blue absorbs red
helium-neon laser light having a wavelength of 632.8 nm and issuing from a flow
cytometer. Therefore, a sample consisting of a number of cells is
instantaneously divided into three groups of spots, i.e., live cells, dead
cells, and cell fractions. The three groups of spots are drawn on an
oscilloscope and allow a ratio between live cells and dead cells to be easily
determined. When Hoechst 33342 and propidium iodide are used to dye DNA of
unfixed cells, live cells and injured cells fluoresce in blue and red,
respectively and can therefore be easily distinguished by a flow cytometer.

US005827742A

United States Patent [19]

Scadden

[11] Patent Number: 5,827,742

[45] Date of Patent: Oct. 27, 1998

[54] **METHOD OF SELECTING PLURIPOTENT HEMATOPIOETIC PROGENITOR CELLS**

[75] Inventor: **David T. Scadden**, Weston, Mass.

[73] Assignee: **Beth Israel Deaconess Medical Center, Inc.**, Boston, Mass.

[21] Appl. No.: **299,902**

[22] Filed: **Sep. 1, 1994**

[51] **Int. Cl.**[6] **C12N 15/06**

[52] **U.S. Cl.** **435/377**; 435/2; 435/325; 435/366; 435/372; 435/375

[58] **Field of Search** 424/43.7, 529; 435/2, 240.1, 240.4, 325, 366, 376, 377

[56] **References Cited**

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[57] **ABSTRACT**

Methods of selecting a population of human cells containing quiescent pluripotent hematopoietic progenitor cells substantially free of mature, human myeloid and lymphoid cells, the quiescent pluripotent progenitor cells obtained by these methods, and methods of using the pluripotent progenitor cells are described.

5 Claims, 5 Drawing Sheets

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DOCUMENT-IDENTIFIER: US 5827742 A
TITLE: Method of selecting pluripotent hematopioetic progenitor cells

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DEPR:
Cells were stained with propidium iodide (PI) and Hoechst 33342 (HO) as
previously described (A. Pollack, G. Ciancio, METHODS IN CELL BIOLOGY, Z.
Darzynkiewicz, H. A. Crissman Eds. (Academic Press,Inc. 1990) vol. 33. Cells
were washed in PBS, resuspended in 100 ul PBS containing 20 .mu.g/ml propidium
iodide (PI) and 10 ug/ml RNase and incubated for 30 min. on ice. Thereafter
1.9 ml of 25% ethanol and 10 .mu.l 1 mM HO-33342 (HO; Sigma, St. Louis, Mo.,
U.S.A.) was added and the cells were incubated for another 30 min. on ice. HO
and PI fluorescence were analyzed using an EPICS 750 series flow cytometer
(Coulter Electronics, Hialeah, Fla.). Fluorescence was excited by a 5 watt
argon ion laser generating 40 mW of light at 351-363 nm. HO emission was
detected through a 450 nm band pass filter. PI emission was detected through a
610 nm long pass filter. Fluorescence from each dye was directed to the
appropriate detectors using a 560 nm short pass dichroic filter. Scattered
laser light was eliminated from the fluorescence detectors by a 380 nm long
pass filter.) followed by flow cytometric analysis on days 0 and 7.

US005423778A

United States Patent [19]

Eriksson et al.

[11] Patent Number: 5,423,778

[45] Date of Patent: Jun. 13, 1995

[54] **SYSTEM AND METHOD FOR TRANSPLANTATION OF CELLS**

[75] Inventors: **Elof Eriksson**, 5 Lanark Rd., Wellesley Hills, Mass. 02181; **Peter M. Vogt**, Newton, Mass.

[73] Assignee: **Elof Eriksson**, Wellesley Hills, Mass.

[21] Appl. No.: **897,357**

[22] Filed: **Jun. 11, 1992**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 707,248, May 22, 1991, Pat. No. 5,152,757, which is a continuation of Ser. No. 451,957, Dec. 14, 1989, abandoned.

[51] **Int. Cl.**[6] **A61F 13/00**

[52] **U.S. Cl.** **604/305**; 604/891.1; 435/240.1

[58] **Field of Search** 604/304-308, 604/890.1, 891.1; 623/15; 424/DIG. 13, 574, 422, 423; 128/888; 435/41, 172.1, 240.1, 240.2, 240.21, 240.23, 240.31, 240.241, 240.243, 287, 289

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(List continued on next page.)

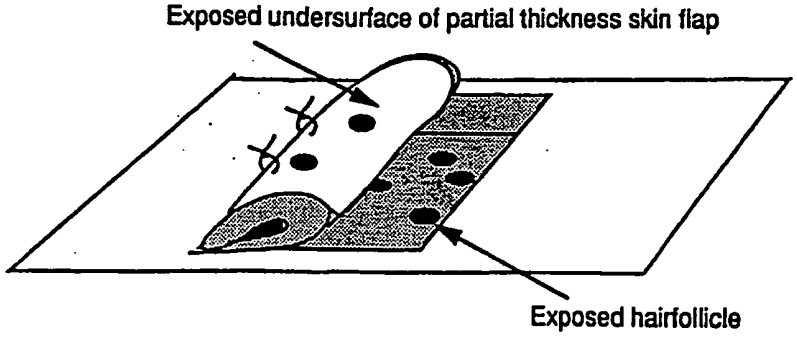
Primary Examiner—C. Fred Rosenbaum
Assistant Examiner—V. Alexander
Attorney, Agent, or Firm—Quarles & Brady

[57] **ABSTRACT**

Gene transfer of genetic material with viral vectors or plasmid, in combination with a wound treatment chamber, into keratinocytes, especially those including a high percentage of epidermal stem cells, has been demonstrated to be particularly effective as a means of implanting genetically engineered cells and obtaining long term survival. By employing the wound chamber system, direct in vivo gene transfer can also be done to exposed cells in an open wound. Skin stem cells which are located in the hair follicles are used to greatly enhance long term survival. The use of the wound chamber system for gene transfer also allows non-invasive assessment of the success of transfer by assaying for the presence of the expressed protein in wound fluid, in contrast to the prior art use of invasive techniques, such as biopsies, in order to achieve the same assessment of early expression. A wide variety of proteins and materials can be expressed, either for secretion into the general blood and lymphatic system, or to alter the properties of the protein, for example, to not express proteins eliciting an immune response against the transplanted cell.

22 Claims, 4 Drawing Sheets

Conceptional partial thickness wound model for in-vivo gene transfer



DOCUMENT-IDENTIFIER: US 5423778 A
TITLE: System and method for transplantation of cells

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ABPL:
Gene transfer of genetic material with viral vectors or plasmid, in combination with a wound treatment chamber, into keratinocytes, especially those including a high percentage of epidermal stem cells, has been demonstrated to be particularly effective as a means of implanting genetically engineered cells and obtaining long term survival. By employing the wound chamber system, direct in vivo gene transfer can also be done to exposed cells in an open wound. Skin stem cells which are located in the hair follicles are used to greatly enhance long term survival. The use of the wound chamber system for gene transfer also allows non-invasive assessment of the success of transfer by assaying for the presence of the expressed protein in wound fluid, in contrast to the prior art use of invasive techniques, such as biopsies, in order to achieve the same assessment of early expression. A wide variety of proteins and materials can be expressed, either for secretion into the general blood and lymphatic system, or to alter the properties of the protein, for example, to not express proteins eliciting an immune response against the transplanted cell.

BSPR:
Gene transfer of genetic material with viral vectors, plasmids, or gene guns into keratinocytes, especially those including a high percentage of epidermal stem cells, in combination with the use of an "in vivo" culture chamber has

been demonstrated to be particularly effective for culture keratinocytes.

DRPR:
FIG. 1 is a schematic of the exposed undersurface of a partial thickness skin
flap used to expose hair follicles to obtain epidermal stem cells. .

DEPR:
The method described herein is based on two principle components. One is the
targeting of epidermal stem cells for gene transfer. The second is the use of
the wound chamber in order to create an in vivo tissue culture environment,
which eliminates the need to culture cells in vitro when introducing the
genetic material into the cells.

DEPR:
Significant expression of both genes in this system was obtained, as shown by
FIGS. 4 and 5a and b. Histologic sections of the skin showed that the
keratinocytes that had the Lac Z marker gene were not stably incorporated into
the basal layer, but migrated to the surface and were lost into the stratum
corneum. When analyzing the reasons behind this, it was concluded that the
dispase separation of dermis from epidermis did not allow for harvesting of a
sufficient amount of stem cells. An additional reason was that the repeated
trauma of harvesting the cells, exposure to tissue culture medium with
relatively high calcium, and the cellular injury from the virus or the plasmid
medium, caused a near terminal differentiation of the keratinocytes.
Accordingly, results were greatly improved by using a higher percentage of
epidermal stem cells obtained from the hair follicles, and decreasing the
exposure to calcium and viral or bacterial medium.

CLPR:

3. The method of claim 2 wherein the cells are selected from the group
consisting of keratinocytes and epidermal stem cells.

CLPR:
4. The method of claim 3 wherein the epidermal stem cells are isolated by
removing the cells from hair follicles on the underside of partial thickness
skin flaps.

CLPR:
13. A method for increasing the yield of genetically engineered cells in a
patient comprising the steps of selecting in vivo epidermal stem cells as the
cells to be engineered and introducing genetic material into said epidermal
stem cells.

CLPR:
21. A method for increasing the yield of genetically engineered cells
implanted in a patient, comprising the steps of selecting epidermal stem cells
as the cells to be engineered, covering the implanted stem cells with a
chamber, and introducing genetic material to the cells within said chamber,
said chamber having an opening securable at the periphery to the skin of the
patient and being formed of a flexible, moisture and gas impermeable material
wherein said chamber contains the genetic material and the cells to be
genetically engineered.

CLPR:
22. A method according to claim 21 wherein the step of selecting epidermal
stem cells includes the step of exposing the basal layer of the patient's
epidermis for the stem cells.